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The Chemical Effects of Gamma Radiation on Blood Sugar

Linda Kay Rames

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THE CHEMICAL EFFECTS OF GAMMA RADIATION
ON BLOOD SUGAR

BY

LINDA KAY RAMES

A thesis submitted
in partial fulfillment of the requirements for
the degree Master of Science, Department of
Pharmaceutical Chemistry, South Dakota
State College of Agriculture
and Mechanic Arts

August, 1960

SOUTH DAKOTA STATE UNIVERSITY
This thesis is approved as a creditable, independent investigation by a candidate for the degree, Master of Science, and acceptable as meeting the thesis requirements for this degree; but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Advisor

Head of the Major Department
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CHAPTER I

INTRODUCTION

The passage of nuclear radiations through materials may cause chemical and physical changes in these materials (14),(25). Particles of ionizing radiation pass some or all of their energy to the electrons of the material and thus cause ionization or excitation. Electrons ejected during ionization may cause secondary ionizations and excitations.

Because of the significance of water in biological and chemical systems, its decomposition by nuclear radiation has been studied (25). Processes of ionization and excitation cause the formation of the free radicals (H) and (OH) from pure water. These may react to form H₂ and H₂O₂. In the case of fast particle radiation, such as that of high energy gamma rays, the number of ionizations per unit of measurement is many times less than that for slow particles. In these cases the (H) and (OH) particles are less concentrated and diffuse into the media to react with the H₂ and the H₂O₂ instead of with each other. If there are traces of halides in the irradiated medium, they react with the (OH) radicals in this manner:

\[ \text{Br}^- + \text{OH} = \text{Br} + \text{OH}^- \]

This inhibits the back reaction of (OH) and (H) to reform H₂O and permits fast particles to produce large quantities of
hydrogen. Also, dissolved oxygen in irradiated water will react with the (H) radicals present to form the free radical \( \text{H}_2^\cdot \). Because of these free radicals, oxidizable or reducible solutes in an aqueous medium may be readily oxidized or reduced.

In a biological system the effects of ionizing radiation on \( \text{H}_2\text{O} \) may ultimately result in cellular disturbances. For example, it can be calculated that a lethal dose of 1000 roentgens affects only one atom out of 2 times \( 10^7 \) atoms (4) (14). It would seem evident, therefore, that some important intracellular material occurring in relatively small quantities is initially affected. These primary effects then conceivably produce other biochemical artifacts with the eventual alteration of cellular function. Indeed, it has been said that acute irradiation sickness is partly due to a basic disturbance caused by ionization which results in cellular enzyme catalysis of the processes of cell synthesis (14).

There have been many studies of the effect of radiation on various constituents of biological systems (5)(7)(19). Because of its extreme importance to the living organism, the effects of ionizing radiation on blood and blood constituents have been of great interest (1)(6)(13)(22). Irradiation of blood generally results in a rise in plasma cholesterol and uric acid, with a decrease in plasma volume, enzymatic activity, and the albumin-globulin ratio. Also noted is the
formation of polysaccharide globules in the capillaries.

Radiation effects on blood glucose have also been noted both in vitro and in vivo (15)(16)(17)(21). Irradiation of both dogs and rats has shown the development of hypoglycemia. One could expect that the effects of ionizing radiation on blood might produce several by-products from blood glucose, since the blood is a complex organ which would yield many other ions and radicals besides those of water upon irradiation. These other substances may alter the process of decomposition or react swiftly with the products.

It is possible that a study of the effect of irradiation on blood sugar and other blood constituents may provide an insight into the etiology of radiation sickness. Since blood acts as the transportation agent for the body and as a solvent for so many complex compounds, it could be the source and carrier of any toxic decomposition products resulting from irradiation. For these reasons the effects of ionizing radiation on blood sugar are the main interest of this study.

Previous research has indicated that furan 2,5-dicarboxylic acid might be one of the irradiation decomposition products of glucose solutions (24)(26)(27). The objective of this study, therefore, is to show whether furan 2,5-dicarboxylic acid is produced upon irradiation of blood.

For this study carbon-14 randomly-labeled radioactive glucose was dissolved in blood serum and the blood serum was
then exposed to gamma radiation from a cobalt-60 source. If the radiation converted the glucose to furan 2,5-dicarboxylic acid, a portion of this acid would be radioactive and could be determined by the carrier technique of analysis.
then exposed to gamma radiation from a cobalt-60 source. If the radiation converted the glucose to furan 2,5-dicarboxylic acid, a portion of this acid would be radioactive and could be determined by the carrier technique of analysis.
CHAPTER II

SURVEY OF THE LITERATURE

Decomposition of Glucose Solutions by Heat

A study of the decomposition of glucose solutions was completed by Webb in 1957 (29). It was concerned with the thermal decomposition of glucose solutions under varying conditions.

Glucose solutions of a wide concentration range were prepared. Some of the solutions were buffered; others were made alkaline or left untreated.

Preliminary findings showed the formation of 5-hydroxymethylfurfural. The reaction was subject to general acid-base catalysis. Results also indicated that the rate of reaction was inversely proportional to the initial concentration of glucose.

Formation of 5-hydroxymethylfurfural would involve 2 - 5 ring closure in the glucose molecule and subsequent dehydration to leave the double bonds.

Decomposition of Glucose Solutions by Gamma Radiation

In a later attempt to solve the problem of prolonging the shelf life of glucose injections, the possibility of sterilization by irradiation was investigated (28). Solutions
of various concentrations and pH were subjected to gamma radiation from a cobalt-60 source. The samples received an approximate dose of 37,860 roentgens per hour.

From this study Webb showed that an acidic product was formed from irradiation of both buffered (0.001 M HCl) and unbuffered 10% glucose solutions. Polarimetric assay of the solutions demonstrated that the percentage of glucose present had varied little. It was evident, therefore, that whatever reaction took place occurred to micromolar quantities of glucose.

The effect of reactant concentration on decomposition using 5, 10, 15, and 20% glucose solutions indicated that the rate of glucose decomposition was approximately linear and was, to some extent, dependent on concentration.

Spectrotransmittance curves of the samples showed a maximum absorption at a wavelength of 260 millimicrons and a minimum absorption at a wavelength of 235 millimicrons. Some of the compounds which could be formed in this way from glucose which would show similar absorption characteristics are dihydroxyacetone, glyoxal, and furfural derivatives.

Chromatography

In a further effort to identify the product or products formed from the irradiation of glucose solutions, Shaw (24) used paper partition chromatography. n-Butanol, glacial
acetic acid and water was the system used and the resulting chromatograms were developed with either ammoniacal silver nitrate or aniline hydrogen phthalate.

A positive identification of reaction products was not possible under these experimental conditions. However, it was possible to eliminate aldohexoses, deoxy-sugars and uronic acids as reaction products.

Ion exchange chromatography was used by Urquhart and Bailey (27) in an attempt to separate the decomposition products. The resins used for the chromatographic column were IRA-4S and IRA-400. Both the hydroxy and chloride forms of each were tried. Separation of the reaction product having absorption in the ultraviolet region appeared successful, as no ultraviolet absorption was observed from distilled water washings of the column. Elution of the columns with either dilute sodium hydroxide or dilute sodium chloride, however, produced drastic changes in the ultraviolet spectrum. Absorption in the shorter wavelengths increased far beyond that in the original, undiluted solution. Maximum absorption at the wavelength of 260 was present only in solutions of acid pH, and absorption in these solutions was greatly diminished. These methods of isolation were, therefore, discarded.

Furan 2,5-dicarboxylic acid

Urquhart (26) compared the ultraviolet absorption
spectrum of furan 2,5-dicarboxylic acid with that of irradiated glucose solutions. He found that the acid showed a maximum absorption at a wavelength of 260 millimicrons similar to that of the irradiated medium. Subsequently he perfected a technique of liquid-liquid extraction of the ultraviolet-absorbing material from the irradiated glucose solutions. Infra-red spectrophotometric analysis of this unknown substance in ether solution gave results which closely resembled the absorption spectrum of furan 2,5-dicarboxylic acid.

On the basis of these results and other information Irquhart proposed that the decomposition product from glucose irradiation showing ultraviolet absorption is furan 2,5 dicarboxylic acid. It is also possible that there are other products formed which do not absorb ultraviolet light.

In summary, his reasons for proposing furan 2,5-dicarboxylic acid as the decomposition product are:

1. The compound is acidic and would account for the drop in pH of the irradiated glucose solutions.
2. The product has a structure which could be derived from oxidative decomposition of glucose.
3. The ultraviolet and infrared absorption data of the decomposition product agrees with that of furan 2,5-dicarboxylic acid.
4. Furan 2,5-dicarboxylic acid does not fall into any
of the groups of compounds previously eliminated by paper chromatographic analysis of the solution.

Carbohydrate Radiation Chemistry

Carbohydrate irradiation has been extensively studied (2)(3)(19)(20)(23)(24)(26)(27)(28)(30). The irradiation of solid carbohydrates causes color and organoleptic changes (30). Besides color changes other effects can be noted from the irradiation of solutions of carbohydrates.

A number of monosaccharides - glucose, d-galactose, d-fructose, and other related compounds such as gluconic acid, gluconic acid lactones, hyaluronic acid, mannitol, and hexosamines - after gamma irradiation in aqueous buffered solution yield a product with an ultraviolet absorption maximum around 264 - 267 millimicrons (18)(24)(26)(27)(28).

Acidification of the irradiated solutions causes the maximum to shift to shorter wavelengths around 245 millimicrons (2). Other known oxidative products and formaldehyde may also be found.

A study of x-ray, ultraviolet, and electron irradiation of glucose solutions was consistent with the above results (2)(3)(20)(23). There was evidence that an unknown acid with a pKa of 4.5 had been formed (3). However, there was evidence that more than one product was being formed (3)(19). Investigation showed that only one of the
products was an acid and paper chromatographic analysis showed that this acid was not glucuronic acid (24). The product which exhibited the ultraviolet absorption was indicated to have a carbonyl group which was fairly resistant to oxidation (3).

Di- and trisaccharide irradiation showed that glycosidic bonds are sensitive to ionizing radiation (2)(18). Aqueous solutions of starch, inulin, maltose, lactose, sucrose, and raffinose showed an ultraviolet absorption maximum at approximately 265 millimicrons after irradiation. Monosaccharides and formaldehyde were also found in the irradiated media. The presence of formaldehyde indicates considerable rupture of carbon to carbon bonds. Evidence showed that the radiation cleaved the 1-2 linkage in sucrose and the 1-6 in raffinose (18).

The irradiation of mucopolysaccharides with ultraviolet light and electrons caused the depolymerization of the large molecules into low-molecular, dialyzable compounds (2). Degradative products from the neutral and alkaline solutions showed an ultraviolet absorption maximum at 267 millimicrons which switched to shorter bands upon acidification. Prolonged ultraviolet irradiation destroyed the absorbing product. This was also true of alkaline glucose solutions.
Furan 2,5-dicarboxylic acid as a Metabolic Product

Furan 2,5-dicarboxylic acid is a normal constituent of human urine (8) (10) (11) (12). Three to five milligrams are excreted per day. The acid has not been found in the urine of dogs, cattle or horses.

A carbohydrate or fat rich diet does not increase the amount of furan 2,5-dicarboxylic acid excreted, but ingestion of glucuronic or galacturonic acids will (12). There is twenty per cent efficiency in the conversion of glucuronic acid to furan 2,5-dicarboxylic acid and sixty-eight percent conversion from galacturonic acid (11).

Upon oral administration of furan 2,5-dicarboxylic acid, only five to six per cent can be isolated from the urine. The biotransformation of the furan 2,5-dicarboxylic acid remains unknown. Using bacteria, however, it can be shown that the substance does not act as an energy source (12). Also, furan 2,5-dicarboxylic acid is not bactericidal and appears to be pharmacologically inactive.

Ketohexoses seem to be the source of furan 2,5-dicarboxylic acid in the body (11). Glucose and glucuronic acid form xylulose in the body (9). The first step in this reaction involves the oxidation of D-glucose to D-glucuronic acid. The D-glucuronic acid form L-gluconic acid; this is converted to L-xylulose, a ketohexose. Ketohexoses can be
dehydrated by the action of sulfuric acid to form furfural derivatives.

In the body furan derivatives are readily oxidized. Aldehyde, ketone, and alkyl substituents on carbons 2 and 5 in the furan ring are oxidized to the carboxylic acid group (11).

The formation of furan 2,5-dicarboxylic acid seems to occur through omega oxidation and ring closure of an aldehyde glucose acid. It is also thought that furan 2,5-dicarboxylic acid may be used for the synthesis of carbohydrates.
CHAPTER III

EXPERIMENTAL WORK

Outline of the Experiment

Following is a general outline of the experimental work involved in this investigation:

1. Blood glucose was labeled by the addition of carbon-14 randomly-labeled glucose to blood plasma.

2. The blood plasma was subjected to approximately one million roentgens of gamma radiation from a cobalt-60 source.

3. A quantity of furan 2,5-dicarboxylic acid was dissolved in the irradiated plasma and a sample of the acid subsequently isolated and purified.

4. The isolated sample of furan 2,5-dicarboxylic acid was subjected to radiocassay.

Separation of Furan 2,5-dicarboxylic Acid

Evidence indicates that micromole quantities of furan 2,5-dicarboxylic acid are formed upon irradiation of glucose solutions. Separation of the acid from glucose solutions has been effected by Uragamiart (26) using a liquid-liquid extraction. However, this method was not suitable for separation of the acid from blood plasma. Centrifugal paper chromatography was, therefore, the first method of separation.
investigated by the writer.

Previous work had not indicated any reagent which would form a colored complex with furan 2,5-dicarboxylic acid and thereby be suitable to use as a developer. Therefore, several common reagents such as silver nitrate, ammoniacal silver nitrate, methyl red, bromophenol blue, and thymol blue were investigated. None of these reagents gave satisfactory results.

An attempt was then made to determine the Rf value of furan 2,5-dicarboxylic acid using centrifugal paper chromatography. Early work with amino acid solutions indicated that the results from centrifugal chromatography were not very reliable. Rate of flow of solvent and speed of revolution were hard to adjust. The solvent front was never uniform. The amino acids were often carried as a steadily widening band rather than as a spot so that determination of Rf values was difficult.

Our procedure was as follows: Normal butanol saturated with acetic acid and distilled water was used as the movable solvent. A Precision Hi-speed Centrifugal Chromatograph was the apparatus used. A small spot of furan 2,5-dicarboxylic acid solution containing 7.5 mg. of acid in 20 ml. of distilled water was placed near the center of a circular piece of Whatman No. 1 chromatographic paper. Setting the revolutions per second at approximately 500, the solvent was
then allowed to flow onto the paper in a steady stream. When the solvent front appeared to be near the edge of the paper, the solvent flow was stopped and the instrument shut off. The solvent front was marked.

Attempts were then made to find a developer to locate the furan 2,5-dicarboxylic acid spot, but they were all unsuccessful.

Since furan 2,5-dicarboxylic acid has a characteristic ultraviolet absorption, the possibility of locating the spot by cutting a strip out of the circle and then cutting the strip into segments which could be individually extracted was considered.

The original furan 2,5-dicarboxylic acid solution was spotted on several small pieces of chromatographic paper. Both distilled water and 0.1 N sodium hydroxide solution were used as solvents for extraction. The ultraviolet absorption of these extractions was determined both before and after acidification with hydrochloric acid. It was decided to use distilled water as an extraction solvent since it gave the most satisfactory results.

Additional chromatograms of furan 2,5-dicarboxylic acid were made. A strip was cut from the chromatogram which included the original spot and the probable path of the acid. The strip was segmented and the segments macerated overnight with distilled water. These extractions showed no ultra-
violet absorption. After further such attempts with negative results, this method of separation was dropped from consideration.

In 1937 and 1945 Flashentrager et al. published papers on the properties of furan 2,5-dicarboxylic acid and methods for separation of the acid from urine (10)(11). Although their work was done with a larger quantity of acid than would be present in our experiments, it was decided to attempt separation of the acid by their procedure.

A modified version of their procedure was tried in an attempt to separate small quantities of furan 2,5-dicarboxylic acid from blood plasma. A sample of hog blood was obtained and the red blood cells were removed by centrifugation and decantation. The blood plasma was refrigerated after addition of sodium oxalate to prevent clotting.

A portion of plasma was acidified with 2% hydrochloric acid and 100 mgm. of furan 2,5-dicarboxylic acid added. The blood plasma solution was successively extracted with small volumes of ether until the total ether volume was approximately three times that of the plasma. The ether extract was then heated to dryness and a few ml. of 50% sulfuric acid added. The mixture was heated to 180° and maintained at that temperature for one hour.

The resulting decomposed mixture was then filtered with hot water rinses and the filtrate evaporated down to a
few ml. which consisted mainly of sulfuric acid. White
crystals precipitated from this acid solution; however, there
was too small a quantity to separate. Attempts to re-extract
the crystals from the acid with ether were unsatisfactory.
After dilution of the acid with distilled water, which caused
solution of the crystals, ether extraction was still unsatis-
factory.

At this time the ultraviolet absorption of the
separated compound was checked after dilution of the acid
containing the crystals from one of the earlier extraction
attempts. There was no ultraviolet absorption detected.

Another extraction was attempted and the ultraviolet
absorption of the acid filtrate was checked immediately.
There was no ultraviolet absorption.

To check the effect of the procedure on furan 2,5-
dicarboxylic acid, known furan 2,5-dicarboxylic acid was
heated at 180°C with sulfuric acid. The mixture was fil-
tered. This filtrate also failed to show ultraviolet
absorption at the proper wavelength or at any wavelength.
It was apparent that the procedure used decomposes furan
2,5-dicarboxylic acid.

The procedure for extraction was rearranged. Known
furan 2,5-dicarboxylic acid was dissolved in blood plasma.
The plasma was heated to dryness and a few ml. of 50% sulfur-
ic acid were added. After heating at 180°C for one hour, the
mixture was cooled, filtered and diluted with hot water to approximately 200 ml. The filtrate was extracted with three times its volume of ether and the ether extract was evaporated on a steam bath.

A water solution of the extract showed an ultraviolet absorption at 265 millimicrons indicating the separation of furan 2,5-dicarboxylic acid.

Synthesis of Furan 2,5-dicarboxylic acid

Furan 2,5-dicarboxylic acid used in the experiment was synthesized following the method of Yoder and Tollens (31).

Thirty grams of mucic acid was heated with 15 ml. of 96% sulfuric acid with stirring over a twenty minute period to 128°C. Then the mixture was heated rapidly to 155°C. The temperature was dropped to 130°C and maintained there for twenty minutes following which the mixture was allowed to cool and 100 ml. of water was added. After heating on a steam bath for ten minutes, the mixture was allowed to stand overnight at 20°C and filtered. Following evaporation of the filtrate the residue was dissolved in hot ethanol. One gram of activated charcoal was added to the alcoholic solution and the mixture was boiled for 15 minutes. The hot solution was filtered until fairly clear and then the filtrate was evaporated. The impure residue was again dissolved in ethanol, boiled with activated charcoal, filtered and evaporated to
The product was recrystallized by dissolving in boiling water, filtering the solution while hot and then allowing to cool with the aid of refrigeration. The crystals which formed were separated by filtration. They were a light brown color.

The ultraviolet spectrotransmittance characteristics of the crystals were determined by means of a Beckman Model DU Spectrophotometer using ultraviolet attachments. Maximum absorption occurred at a wavelength of 265 millimicrons which coincides with the ultraviolet absorption characteristics of furan 2,5-dicarboxylic acid.

Further spectrochemical analysis on the product in the infrared region was performed using the potassium bromide pellet technique. The absorption characteristics of the product were determined over the wavelength range of 2 - 15 microns using a Beckman Model IR - 5 Infrared Spectrophotometer. The spectral-transmittance curve obtained is shown in Figure 1. Comparison of these characteristics with those of furan 2,5-dicarboxylic acid, as well as with the vibrational absorption spectra of known structural components indicated that the product was furan 2,5-dicarboxylic acid and suitable for use.
Figure 1. Integrated Spectrum of Furan-3,2-dicarboxylic acid
Irradiation of Blood Plasma

Rabbit blood plasma was chosen as the medium on which the effects of gamma radiation on blood sugar would be studied. The blood was obtained by opening the thoracic cavity of a rabbit and cutting the aorta. The blood was collected in a beaker containing a heparin solution to inhibit clotting. After centrifuging to pack the red blood cells, the blood plasma was decanted and kept under refrigeration. It was noted that the blood had hemolyzed to a considerable degree and that the plasma contained free hemoglobin.

A total of 9.01 mg. of radioactive D-glucose randomly-labeled with carbon-14 was added to 8 ml. of the blood plasma contained in a small beaker. The specific activity of the radioglucose was 1 milliCurie per millimole, and a total activity of 50 microcuries was present.

After solution was evident, the blood plasma was poured into a half-ounce prescription bottle. The beaker and contents were rinsed twice with approximately four ml. portions of blood plasma and the rinsings were combined with the original solution in the half ounce bottle.

For irradiation, the bottle containing the labeled blood plasma was fastened to a peg board constructed in such a manner that the cobalt-60 irradiation source could be positioned at a known distance by remote control. The source was placed exactly three inches from the bottle.
The cobalt-60 source emitted a gamma ray activity of approximately 103,6 curies. At a distance of three inches the blood plasma received an approximate dose of 23,760 roentgens per hour or a total of approximately 1,230,000 roentgens in the 50.5 hours of irradiation.

The Carrier Technique of Radiоchemical Analysis

The irradiated plasma was poured into a large evaporating dish and a small amount of distilled water used to wash out the bottle. A 100 mgm. portion of synthesized furan 2,5-dicarboxylic acid was then added to the plasma. When the acid was added, a noticeable change in the physical characteristics of the plasma occurred with the formation of a pulpy, gray precipitate. The precipitate could have been protein thrown out of solution due to the acidity of the added furan 2,5-dicarboxylic acid, however this was not determined.

Following the procedure for isolation of furan 2,5-dicarboxylic acid developed in the preceding section, the blood plasma was heated to dryness. During the heating period the air in the hood was monitored, but there was no evidence of carbon dioxide or other volatile radioactive decomposition products.

When the plasma was reduced to a brownish gray dry
mass, the evaporating dish was cooled and ten ml. of 50% sulfuric acid was added to the residue. After heating at 180°C, for one hour, the resulting mixture was cooled and diluted with distilled water. The diluted acid mixture was then filtered to remove the carbon and other insoluble particles. Two portions of a 1:1 ethanol-water solution were used to rinse the evaporating dish and wash the filter.

The filtrate was heated to remove some of the ethanol in which the furan 2,5-dicarboxylic acid is very soluble. When the volume was reduced to 100 ml., approximately one-half, heating was stopped and the solution cooled.

The acid filtrate was successively extracted with 75 ml. portions of ether. A total of 525 ml. of ether was used for the extraction. The ether extracts were placed in an evaporating dish on a small heating plate to speed evaporation.

The ether residue was then dissolved in 50 ml. of hot alcohol and the solution was filtered. The alcoholic solution still had a slight brown color after filtration.

The alcoholic solution was evaporated down to a few ml. and placed in a tared counting planchet following which the solution was evaporated to dryness under infrared heat. Ethanol used to rinse the beaker from which the solution came was added to this residue and it was again evaporated to dryness. The residue was a dirty brown color.
A radioassay of the residue was performed using a windowless gas counter. The weight of the residue assayed was 58.7 mgm. and the activity 89,456 counts per minute corresponding to a specific activity of 1524 counts per minute per milligram.

The planchet was then placed in a beaker and the residue was dissolved in hot alcohol. Activated charcoal was added to the solution and it was brought to a boil. The solution was filtered hot giving a relatively clear filtrate which was evaporated and radioassayed in the same manner as the first sample. The residue was composed of white crystals. The weight of the sample was 11 mgm. with an activity of 14,424 counts per minute corresponding to a specific activity of 1311 counts per minute per milligram.

The residue was purified a third time. The weight of the final sample was 10.7 mgm. and its activity 14,775 counts per minute corresponding to a specific activity of 1331 counts per minute per milligram.

Distilled water was used to dissolve the final residue. The ultraviolet absorption characteristics of this aqueous solution were determined by means of a Beckman Model DU Spectrophotometer with ultraviolet attachments using a distilled water standard. The results agreed with the ultraviolet characteristics of the decomposition product from the irradiation of glucose solutions and synthesized furan.
2,5-dicarboxylic acid.
CHAPTER IV

DISCUSSION

Though furen 2,5-dicarboxylic acid was isolated as a metabolic product by Flashentragcr et al. (10) as early as 1937, its physiological action and biochemical transformation have not been extensively studied.

Research (8) (11) has indicated that the formation of furen 2,5-dicarboxylic acid in a biological system is dependent on the presence of glucuronic acid or galacturonic acid rather than that of glucose. This indicates that the first step in the metabolic formation of furen 2,5-dicarboxylic acid from glucose would require oxidation to glucuronic acid or rearrangement and oxidation to galacturonic acid.

The formation of radicals from the ionization of water provides a medium in which oxidative and reductive processes are highly probable.

The formation of radicals during the irradiation of blood would be enhanced by the presence of halide ions. The complexity of blood would lead to the formation of ions and other radicals besides (II) and (0III).

Evidence indicates that the second step in the formation of furen 2,5-dicarboxylic acid would be formation of a ketohexose by further oxidation or rearrangement of glucuronic acid (11).
Ring closure of the ketohexose would yield a heterocyclic ring containing oxygen with substituents on carbons 2 and 5. Dehydration of this structure by removal of two hydrogen and two hydroxyl groups would yield the furan ring. Evidence has already been shown proving that oxidation of the substituents on carbons 2 and 5 of the furan ring occurs readily (11).

Under the conditions of our experiment it is apparent that furan 2,5-dicarboxylic acid is a decomposition product of blood glucose upon irradiation of blood plasma.

The carrier technique of radiochemical analysis is accepted today as one of the better ultra-micro methods of qualitative organic biochemistry. Essentially the method involves the biosynthesis of the suspected compound using radioactive precursors. The compound biosynthesized would then have the radioactive atoms incorporated into its structure and can be identified in a complex mixture because of this physical property by the use of radiation detection equipment. In order to differentiate from other radiochemicals that might be present, however, the suspected compound is isolated from the mixture. The term carrier used for this procedure signifies non-radioactive material, identical chemically to that of the suspected product, which is added to the system and then recovered. If the recovered material is radioactive after purification, then a proof exists for the
formation of that product in the original system.

The presence of radioactivity in the sample of furan 2,5-dicarboxylic acid indicates beyond a doubt that this product resulted from a transformation of the radioglucose added to the blood plasma.

The difference in activity of the purified and unpurified samples was probably due to large quantities of degradative fragments of radioglucose and possibly other organic substances formed by the action of the concentrated sulfuric acid. Since other radioactive compounds can be formed in the extraction procedure, it is essential that the furan 2,5-dicarboxylic acid isolated be purified to a constant specific activity, in order to state that the activity is due mainly to radioactive furan 2,5-dicarboxylic acid. It can be seen from a comparison of the specific activities of the second and third isolations (1311 cpm/mg, versus 1381 cpm/mg) that purification of the furan 2,5-dicarboxylic acid was adequate for our conclusions. In addition the ultraviolet absorption characteristics of the isolated product agreed with those of synthesized furan 2,5-dicarboxylic acid.

Since furan 2,5-dicarboxylic acid has been shown to be physiologically inactive(10), its formation from glucose by irradiation would not seem to be a toxic reaction. Extensive formation might decrease the amount of glucose present in the
blood and be one of the contributing factors responsible for
the hypoglycemia often present in animals after irradiation.
CHAPTER V

CONCLUSIONS

1. A study of a chemical effect of gamma radiation on blood sugar using the radioactive tracer technique has been made.

2. Furan 2,5-dicarboxylic acid was shown to be one of the decomposition products formed from gamma irradiation of blood sugar.

3. The procedure for separation of furan 2,5-dicarboxylic acid from urine can not be used for separation of the acid from blood due to some hindrance in the initial step of ether extraction.

4. A further study of the effect of irradiation of blood on blood sugar with radioactive glucose would be feasible to prove the identity of other decomposition products.
LITERATURE CITED


